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Automated analysis of $^{13}C/^{12}C$ ratios in CO_2 and dissolved inorganic carbon for ecological and environmental applications

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Stable carbon isotope ratios ($^{13}C/^{12}C$) are a valuable tool for studying a wide range of environmental processes, including carbon cycling and subsurface microbial activity. Recent advances in automated analysis provide the opportunity to increase greatly the ease and consistency of isotopic analysis. This study evaluated an automated headspace sampler linked to a commercially available CO_2 preconcentration system and continuous flow isotope ratio mass spectrometer. Field sampling and analysis methods are illustrated for $\delta^{13}C$ of soil respired CO_2, from both tracer and natural abundance experiments, and dissolved inorganic carbon from contaminated groundwater. The automated system demonstrated accuracy, precision, and linearity, with standard errors below 0.1‰ for replicate gas standards run at concentrations varying five-fold. It measured 40 samples per 10-hour run, with concentrations ranging from ppb to percentage levels. In the field, gas samples were injected into nitrogen-filled autosampler vials, thereby allowing use of small sample volumes, control of analyte concentration, and direct analysis by the automated system with no further preparation. A significant linear relationship between standard concentrations and peak area allows for accurate estimates of sample CO_2 concentration from the mass spectrometric data. The ability to analyze multiple small-volume samples with minimal off-line preparation should enhance the application of isotopes to well-replicated field experiments for process-level studies and spatial and temporal scaling. Copyright © 2003 John Wiley & Sons, Ltd.

Stable isotope signatures are a powerful tool for investigating soil carbon processes and ecosystem-atmosphere exchange of trace gases such as CO_2.[1–10] In vadose zone pore water and groundwater, the isotopic compositions of dissolved organic and inorganic carbon compounds are indicators of subsurface biological activity including degradation of organic contaminants such as petroleum hydrocarbons[11–15] and chlorinated solvents.[16–19]

Historically, cumbersome sampling and preparation techniques have restricted the application of stable isotope measurements for field research. Analyses of ambient concentrations of target compounds such as CO_2 required relatively large samples. In addition, time-consuming off-line preparation techniques limited the number of samples that could be analyzed. Recently, a number of automated biomedical and atmospheric carbon stable isotope analytical systems have become commercially available. As a result, it is now possible, with minor modifications of commercially available equipment, to achieve rapid and automated analysis of C isotopic ratios in soil CO_2 and dissolved inorganic carbon (DIC), with a system that is compatible with simple field sampling procedures.

For an automated ^{13}C analysis system to be viable, five requirements must be satisfied: (i) reported $\delta^{13}C$ values of the analyte must be reproducible, i.e. analysis must be precise; (ii) reported $\delta^{13}C$ values of the analyte must be correct relative to an international standard, i.e. analysis must be accurate; (iii) reported $\delta^{13}C$ values of an analyte must not significantly alter with changes in analyte carbon concentration, i.e. analysis must be linear; (iv) samples should be analyzed rapidly with minimal off-line preparation, i.e. have rapid throughput; and (v) the automated system should be compatible with straightforward field sample collection.

Field collection methods should produce readily analyzed samples from a wide range of environmental conditions and CO_2 concentrations. For example, CO_2 concentrations can range from sub-ambient (<370 ppm) to more than 2% in the top meter of soil. Ideally, the sampling technique should work when a field site's elevation (and ambient pressure) differs

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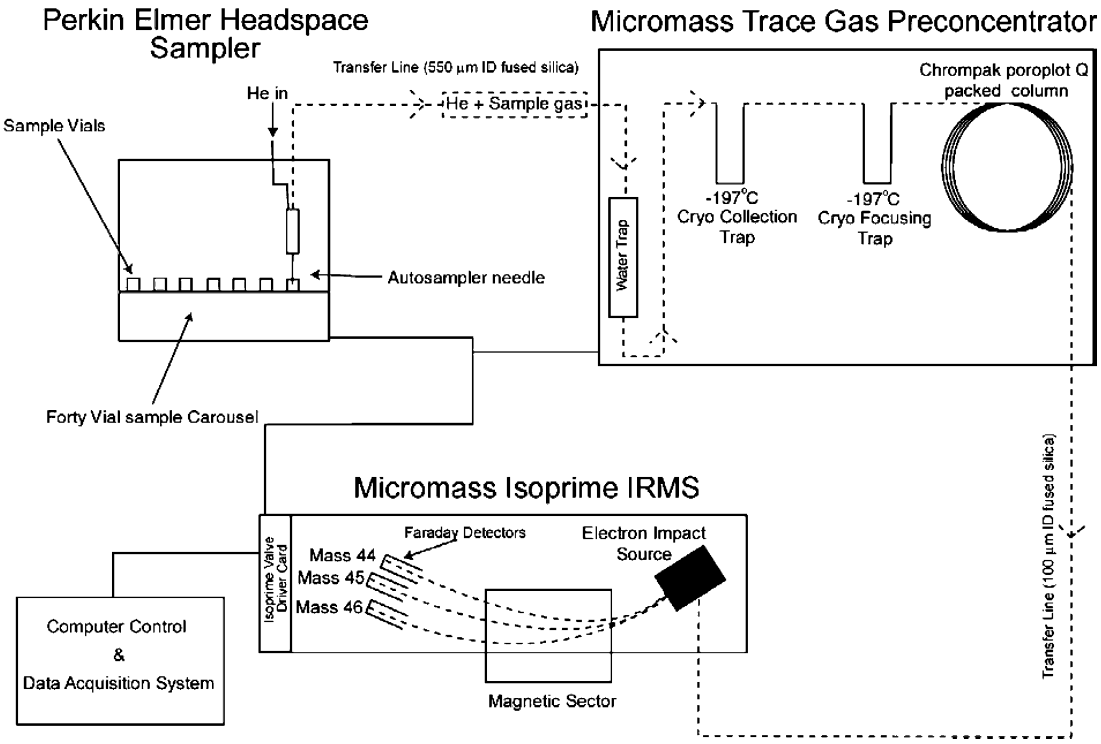

Figure 1. Schematic of the automated system, consisting of a Perkin Elmer headspace sampler linked to a Micromass cryo-focusing system and Micromass Isoprime IRMS.

from that of the laboratory. Chiefly, procedures are needed that require a small volume of sample collected in a way that does not cause isotopic fractionation during collection and minimizes disturbance of the system being studied.

Our objectives were (1) to test the performance of a commercially based system for rapid, automated analysis of ^{13}C in CO_2 and DIC and (2) to develop field sampling procedures that minimize disturbance and off-line preparation while allowing for accurate measurements and rapid throughput to support replicated field studies.

We linked a headspace sampler to a cryo-focusing system, which in turn was connected to an isotope ratio mass spectrometer (IRMS; Fig. 1). Similar analytical systems are available from the manufacturers Micromass, PDZ Europa, and ThermoFinnigan, although the capacity for software linkages to a headspace sampler and the ability to measure sub-ambient CO_2 concentration differs among instruments.

To test system performance with field samples, soil gas and water samples were collected from three sites, representing natural abundance and ^{13}C-enriched sources (Table 1): Jasper Ridge Global Change Experiment (hereafter Jasper Ridge), Blodgett Forest Decomposition and Humification Experiment (hereafter Blodgett Forest), and a shallow groundwater site at the Lawrence Berkeley National Laboratory (LBNL) contaminated with chlorinated solvents (hereafter LBNL site).

Table 1. Isotopic signatures of carbon in two test cases. For Jasper Ridge, the season-average $\delta^{13}C$ of the elevated CO_2 atmosphere was estimated from the $\delta^{13}C$ of plants grown in that atmosphere and assuming that plant material C is 20‰ more negative than atmosphere (i.e., plant discrimination $\Delta = 20‰$ based on the control plots and Ref. 26)

Carbon source	Mean $\delta^{13}C$ (‰)
Atmospheric CO_2	−8.5
Jasper Ridge Global Change Experiment	
Atmospheric CO_2 elevated with fossil fuel derived CO_2	~ −26.8
Plant carbon fixed from ambient atmospheric CO_2	−28.3
Plant carbon fixed from ambient CO_2 elevated with fossil fuel derived CO_2	−47.8
Blodgett Decomposition Experiment	
Native soil organic matter	−25.7
Native *Pinus ponderosa* needles	−27.3
^{13}C-enriched *Pinus ponderosa* needles	+2488

METHODS

The analysis system

Headspace sampler linked to trace gas preconcentrator and IRMS

The system for automated analysis of $\delta^{13}C$ in CO_2 and DIC was made by linking a Perkin Elmer headspace sampler, model HS 40 (HS40), to a Micromass trace gas preconcentrator (TG) and Micromass JA series Isoprime isotope ratio mass spectrometer (Isoprime IRMS; Fig. 1). The trace gas system enables analysis of µL to mL volumes of analyte with chromatographic separation of potential interference from N_2O. The Isoprime operating and data acquisition system control the trace gas and HS40. The system is housed at the Center for Isotope Geochemistry (CIG) at LBNL.

The HS40 extracts aliquots of gas samples from crimp-seal vials. Its automated carousel holds forty 22-mL vials. When prompted by the Micromass operating system, the HS40 punctures a vial with a hollow needle. An aperture at the point of the needle enables a stream of He to pass from the

HS40 into the sample vial. The He flow is subject to 15 psi and raises the pressure within the sample vial to 15 psi. Once the vial is pressurized, the flow of He to the sample vial is halted and a valve connects the aperture of the needle to a transfer line to the TG inlet manifold. The transfer line carrier gas is He flowing at 10 psi. Due to the difference in pressures between vial and carrier gas, a portion of the He carrying the sample gas is forced into the transfer line where it is carried to the TG inlet manifold and merges with the TG He carrier gas flow. The pressure of the carrier gas within the TG is set at 2.5 psi, resulting in negligible flow resistance between the HS40 transfer line and the TG inlet manifold.

As shown in Fig. 1, within the TG, the sample is passed through a magnesium perchlorate trap to remove H_2O. The CO_2 (and other condensable compounds) are preconcentrated in a cryo-trap at liquid N_2 temperatures. After 240 s, the trap is heated to 80°C, vaporizing the compounds collected in the trap. The compounds are then transferred and cryogenically focused into a second liquid N_2 cryo-focusing trap. After an additional 240 s, the cryo-focusing trap is heated and the volatile components of the sample passed through a Nafion membrane (to remove any remaining H_2O) and injected onto a PoroPlot Q packed gas chromatography (GC) column. The PoroPlot Q column separates CO_2 from N_2O gas streams. The two compounds have the same mass/charge ratio and are thus indistinguishable by analysis in the IRMS. Although N_2O concentrations in atmospheric samples are negligible, soil gases can contain quantities of N_2O large enough to significantly alter $\delta^{13}C$ measurements (e.g., Revesz and Coplen[28]). As the sample is eluted from the GC column, it is transferred to the Isoprime via a 1.5-m length of 100 μm i.d. deactivated fused-silica tubing.

Sample vial preparation

The vials used for sample collection and storage were 22-mL Perkin Elmer headspace sample bottles (B010-4236), sealed with 20 mm butyl septa (Supelco, 27232) and aluminum open center seals (Supelco, 2730). Other vials are also compatible with the Perkin Elmer headspace sampler. To prepare the vials for sampling, they were filled with N_2 gas (99.9995%) by placing them inside a disposable PVC glove-bag (Aldus Z112828) that is evacuated and filled with N_2 three times. The sample bottles were sealed inside the N_2 atmosphere of the bag (this technique was adapted by Tu *et al*.[25]). For DIC samples, prior to placing the vials in the glove-bag, 0.5 mL of 85% orthophosphoric acid (H_3PO_4) was added to each sample bottle.

Calibration of the Isoprime IRMS

Internal CO_2 standards

During each individual analysis, a pulse of analytical-grade pure CO_2 reference gas is injected into the Isoprime via the reference gas box (Fig. 1). The $^{13}C/^{12}C$ ratio of this gas is determined independently by analysis with the CIG VG Prism series II dual-inlet isotope ratio mass spectrometer (Prism) and calibrated against the international standard Vienna Pee Dee Belemnite (VPDB). The Isoprime data acquisition system calculates the δ^3C value of the unknown by comparing the ratio of the standard gas to that of the sample. All values are reported using the delta (δ) notation with per mil (‰) variations relative to VPDB, as described in Eqn. (1).

$$\delta^{13}C(‰) = \frac{\left(^{13}C/^{12}C\right)_{sample} - \left(^{13}C/^{12}C\right)_{VPDB}}{\left(^{13}C/^{12}C\right)_{VPDB}} \times 1000‰ \quad (1)$$

Correction for background contribution to results

There are several sources of background CO_2 contamination in analyses. The mass spectrometer and automated system have a trace background CO_2 level. The initial vial preparation is not able to remove all CO_2 from the vials. There may also be a small amount of leakage during sample storage. For smaller samples this background could result in significant shifts in the measured $\delta^{13}C$ values. To correct for this shift, 'blank' vials are prepared with every batch of sample vials. The blank vials are analyzed in the same manner and at the same time as samples. The amount and $\delta^{13}C$ value of the gas in the blank vials are subtracted from the sample $\delta^{13}C$ using the mass balance subtraction described in Eqn. (2).

$$\text{Corrected sample } \delta^{13}C = \frac{\delta_S \cdot A_S - \delta_B \cdot A_B}{(A_S - A_B)} \quad (2)$$

where δ_S = uncorrected $\delta^{13}C$ of sample peak, A_S = peak area of sample, $\delta_B = \delta^{13}C$ of background peak and A_B = peak area of background. The blank corrections require the ability to measure sub-ambient or small-volume samples, even if the field samples have high concentration. The typical background of the automated system in these tests was equivalent to a vial concentration of approximately 10 ppm CO_2. After a 1-week storage period, typical background levels for the vials plus autosampler system were equivalent to 20 ppm CO_2 vial concentration. At present the only way to measure such small amounts of carbon is with a system that preconcentrates the sample.

External CO_2 standards

To ensure that the process of sample collection, storage, and analysis does not fractionate the isotopic composition of the sample gas, an external process standard is analyzed with every batch of samples. For gas samples, the standard is an 80-L tank of 2% CO_2 in N_2 (2% STD). A set of standard vials were made, carried to the field with the sample vials, and analyzed at the beginning and end of the IRMS sample run. For DIC samples, the analyses are compared with DIC in the deionized water supply at LBNL (Berkeley tap water, or BTW). The measurements reported for reproducibility were performed using a single aliquot of water. There are variations in the $\delta^{13}C$ values of different water aliquots, but they are relatively small (<1‰). The $\delta^{13}C$ values for these standards were determined by off-line separation and purification of the CO_2 or DIC followed by analysis on the Prism.

Precision, accuracy, and linearity

To assess precision and accuracy, ten replicate CO_2 standards (2% STD) at the same analyte concentration were run on the autosampler system and the dual-inlet Prism. The process was repeated for DIC analysis with BTW standard. To test system linearity, five pairs of CO_2 and DIC samples were analyzed at 100 ppm increments from 100 to 500 ppm (Table 3).

These analyte concentrations correspond to field CO_2 concentrations of 200 to 1000 ppm, because field samples are diluted by one-half when injected into the vial, as explained below.

Field sample collection and analysis of soil gases and water

Field sites

Jasper Ridge Global Change Experiment is a factorial manipulation of CO_2, temperature, precipitation, and nitrogen levels in an annual grassland in California,[20] using free air carbon enrichment (FACE). The elevated CO_2 treatments are created by adding CO_2 from fossil fuel to ambient air. The fossil CO_2 is depleted in ^{13}C relative to the ambient atmosphere so plant biomass grown under elevated CO_2 has lower $\delta^{13}C$ values than does plant biomass grown under ambient conditions. This difference creates an isotopic marker for plant C inputs and soil organic matter fixed before versus after the elevated CO_2 treatment started (Table 1). Consequently, the isotopic signature of respiration in elevated and ambient CO_2 plots can be used to quantify decomposition and respiration fluxes of new versus old organic carbon (i.e., where old refers to C fixed before the experiment began), and to assess the effect of multiple global change treatments on these fluxes. For this study, the flux and carbon-isotope signature of soil respiration from control and elevated CO_2 plots were used to determine the carbon sources for respiration.

Blodgett Forest Decomposition and Humification Experiment is a 5-year field study testing the effects of soil depth (O vs. A horizon) and litter type (fine roots vs. needles) on decomposition rates, humification rates and pathways in a temperate forest soil. In November 2001, ^{13}C- and ^{15}N-labeled *Pinus ponderosa* litter types were placed in O and A soil horizons in an 80-year-old conifer forest in the Sierra Nevada, California. Because the added litter is isotopically enriched compared with native soil carbon, the isotopic signature of soil-respired CO_2 reveals *in situ* decomposition and efflux of the added litter.

The groundwater LBNL site is contaminated with chlorinated solvents including perchloroethene (PCE), trichloroethene (TCE) and 1,1,1-trichloroethane (TCA). There is strong evidence that natural microbial activity is degrading some of these contaminants, but the mechanisms and extent of biodegradation are not clear. As part of a study to assess the degree of intrinsic bioremediation of chlorinated solvents at the site, the carbon isotope compositions of DIC in groundwater samples were measured. In aerobic environments, the $\delta^{13}C$ values of DIC are similar to the $\delta^{13}C$ values of the carbon source. In anaerobic environments (especially under methanogenic conditions), DIC will be significantly enriched in ^{13}C relative to the source. Different redox conditions favor different microbial mechanisms for biodegradation of chlorinated compounds. The $\delta^{13}C$ values of DIC from two sets of samples collected from the site were analyzed; one using conventional off-line preparation techniques and the second using the system described in this paper.

CO_2 field sampling procedure

We made field measurements to determine the $\delta^{13}C$ value of soil-respired CO_2 in two steps. For both steps, we used a LI-COR Li-6400 portable infrared gas analyzer (IRGA; LI-COR, Lincoln, NE, USA) and a 6400-9 soil respiration chamber that we modified for headspace gas collection (basal area 180 cm^2, placed on a permanent PVC base). In the first step, we collected samples of headspace gas for isotopic analysis at five time points. The incubation began at ambient CO_2 concentration and samples were taken at 50 ppm intervals, over roughly 10–15 min depending on flux rate. The CO_2 concentration at each time point, as determined by the Li-6400, was noted. The Li-6400 circulates air into the IRGA with a small fan so CO_2 concentration can be determined without using a pump or otherwise disturbing the soil gas flux. Second, we measured total soil respiration, after airing out and replacing the chamber.

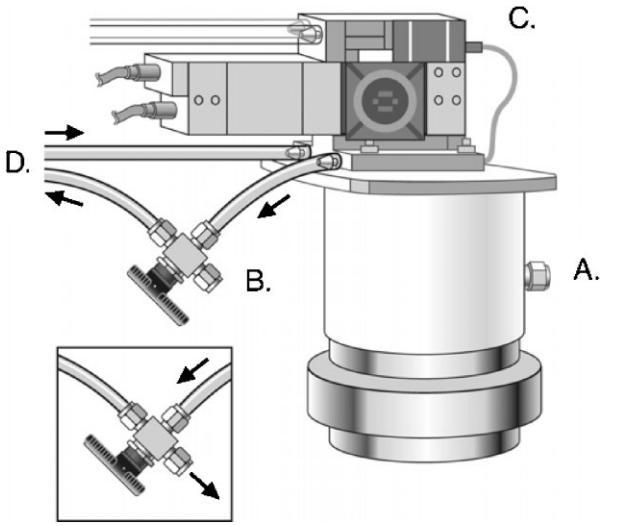

Figure 2. Schematic of the Li-Cor soil respiration chamber modified for gas sampling for isotopic analysis. A: Swagelok fitting on the side of chamber; B: three-way valve, in flux sampling and (inset) gas aliquot sampling positions; C: infrared gas analyzer sensor head; and D: hoses to scrubber and pump in Li-COR console.

Gas samples for isotopic analysis were collected with a needle attached with epoxy or silicone to a glass syringe, inserted through a septa connected to the chamber headspace. To install a port for the septa, we tested two alternative chamber modifications (Fig. 2). The first approach was to plumb a three-way valve in the tubing leading from the chamber to the IRGA soda lime CO_2 scrubber (stainless steel Whitey 3-way valve, Swagelok no. ss-42xS4, with Teflon or Nylon front ferrules) and place a septa into the third opening. One valve position connects the chamber to the septa for needle and syringe entry. The other position connects the chamber to the scrubber in the LI-COR console for respiration measurements. The second approach for adding a sampling port was to drill an opening into the side of the chamber wall and epoxy a Swagelok fitting, with septa, so that a needle could sample the headspace air directly. The latter method eliminated dead air volume. Using either modification, the syringe was plunged gently to mix the line air with the headspace before gas collection.

After collecting a sample, the syringe was sealed by putting the needle into a butyl rubber stopper until the sample was injected into the vial, a maximum delay of 20 min. Just prior to

injecting the sample into the vial, a syringe was used to remove gas from the vial. Specifically, a volume of vial atmosphere (N_2) equal to the volume of sample to be injected was removed. Thus, after the sample was injected the internal vial pressure was near atmospheric, to minimize potential for leakage or contamination.

This method of filling vials helps avoid problems that may accompany the use of evacuated vials or flasks that are flushed completely with sample during collection: maintaining desired pressure, keeping analyte concentrations relatively constant, and requiring only a small sample. As an example of the first problem, consider sample collection at high elevation (low air pressure) sites. At the Sierra Blodgett site (1350 m elevation), we adjusted the purge and sample volumes (12% less gas was withdrawn from the vial than was added of sample), such that the resulting vials were at ambient pressure in the laboratory near sea level.

To avoid analytical errors caused by analyte concentrations that are outside of the linear range for the mass spectrometer, the ability to inject different volumes allows the concentration of CO_2 in the vial to be controlled easily. Sample volumes ranged from 10 μL (for the almost pure CO_2 inlet to the elevated CO_2 plots) to 10 mL (for chamber headspace near ambient concentrations). If desired, the volume of each successive syringe in an incubation can be decreased as headspace CO_2 increases, so that all vials for analysis have the same CO_2 concentrations. The amount of N_2 withdrawn from the vial depends on the sample volume to be injected and the desired vial pressure. The vials were analyzed with the autosampler directly from the field, with no off-line preparation.

Soil respiration rates were measured right after collecting the isotope samples. The 6400-9 chamber, like many closed static chambers, has a small opening to prevent pressure disequilibria that ruin flux determinations. This hole should be plugged for isotope sampling, particularly where elevated CO_2 inlets or operator breath could be pulled into the chamber when gas samples are extracted. We used a locking quick-connector from Small Parts, Inc. that can be coupled or uncoupled as needed and that screwed into the standard LI-COR pressure equalization port (brass quick connector, double shut off, 10/32″ thread, Y-21700-DS). An alternative approach to closing the opening is to place a balloon over the equalization port *inside* the chamber. This has the advantage that balloon expansion helps prevent soil air from being drawn into the chamber when gas samples are withdrawn (syringe volumes represent less than 1% of the chamber volume).

Sample collection and laboratory preparation of DIC

Water samples for DIC analyses were collected in the field and immediately sealed in sample vials (preferably amber glass EPA vials) with no headspace in order to minimize any exchange of CO_2 with the headspace atmosphere. If the samples were not processed within 3 days, they were stored at 4°C until being processed. For analysis on the mass spectrometer, the sample was injected with an SGE locking syringe (SGE 031905) through the septa of the HS40 vials containing H_3PO_4. The amount of sample varied according to the concentration of DIC in the sample, but was generally between 50–1000 μL of water. After injection, the sample was shaken to mix the water with the acid, taking care not to get the liquid on the septa (vortex mixers are ideal). We compared analyses of $\delta^{13}C$ values of groundwater DIC by the automated system versus by off-line preparation and dual-inlet mass spectrometry. For the latter, a 40-mL reaction vessel containing 3 mL of H_3PO_4 was sealed with a septum and pumped out through a needle attached to a vacuum line. 1–10 mL of sample were injected into the reaction vessel, acidifying the water and converting the DIC to CO_2. The reaction vessel was then re-attached to the vacuum line and the evolved CO_2 was pumped out of the vessel, separated from accompanying water vapor using a series of cryogenic traps, and analyzed on the Prism. The standard reproducibility for this technique is ±0.5‰.

Field data analysis

The $\delta^{13}C$ of soil CO_2 efflux was estimated according to the Keeling plot method.[9,21] Specifically, the $\delta^{13}C$ signature was calculated as the y-intercept of the linear regression of $\delta^{13}C$ vs. $[CO_2]^{-1}$ for the five data points per incubation.

In soil respiration with two isotopically distinct sources of carbon, the relative contribution of each source to respiration can be calculated from a simple mass balance of the isotopic signatures,[22] as shown in Eqn. (3):

$$\text{Fraction contributed by source 1} = \text{F} = \frac{\delta_T - \delta_2}{\delta_1 - \delta_2} \qquad (3)$$

where $\delta_T = \delta^{13}C$ of total soil respiration, $\delta_1 = \delta^{13}C$ of respiration of source 1 and $\delta_2 = \delta^{13}C$ of respiration of source 2. When source 1 is new or experimentally added C, F gives the fraction of new C in total respiration. In the examples presented here, $\delta_T = \delta^{13}C$ of soil respiration in the experimental plot; $\delta_2 = \delta^{13}C$ of respiration of native (or old) soil C; and $\delta_1 = \delta^{13}C$ of respiration of the isotopically labeled litter (Blodgett) or plant inputs fixed under elevated CO_2 (Jasper Ridge).

Neither δ_1 nor δ_2 can be measured directly since they co-occur. However, δ_2 can be estimated as the $\delta^{13}C$ of soil respiration from control plots (native organic matter), given the assumption that respiration of the native organic matter has the same isotopic signature under control and experimental conditions. There are two ways to estimate δ_1. It can be approximated by the $\delta^{13}C$ of the labeled inputs (i.e., by assuming that respiration has the same signature as the substrate). The second method takes into account that the isotopic signature of respired C is not exactly the same as the plant substrate. There may be a 1–2‰ offset between fresh plant litter and soil organic matter below 5 cm in soil.[23] In addition, fractionation by the microbial agents of respiration has not been quantified. Where differences of 1‰ are significant for the mass balance, the fractionation between plant inputs and respiration can be measured in the control plots, and this delta difference added to the new carbon substrate to get the respiration term. This approach is shown for Jasper Ridge below.

RESULTS

System precision, accuracy, and linearity

The replicate analyses of the 2% STD gave an average value of −36.6‰ with a range of 0.9‰ and standard error of 0.06‰

Table 2. Accuracy and precision of the trace gas IRMS ^{13}C analysis compared with replicate analysis performed by a dual-inlet IRMS system. Means are not significantly different by t-test at 0.05 confidence level

System used for analysis	Standard	n	Mean $\delta^{13}C$ value (‰)	Standard deviation	Standard error
Automated trace gas IRMS (Micromass Isoprime)	2% STD	10	−36.62	0.20	0.06
Dual-inlet IRMS (VG Prism 2)	2% STD	10	−36.50	0.02	0.01

Table 3. Linearity. Ability of the trace gas IRMS system to determine $\delta^{13}C$ and total CO_2 concentration of samples containing different concentrations of CO_2

CO_2 concentration (ppm)	n	Mean IRMS ^{12}C peak height, 2%STD (10^{-8} amps)	Mean $\delta^{13}C$ 2% STD (‰)	Mean $\delta^{13}C$ BTW (‰)
100	2	1.84	−36.58	−9.82
200	2	3.52	−36.55	−9.87
300	2	5.32	−36.63	−9.96
400	2	7.08	−36.66	−9.85
500	2	8.9	−36.57	−9.93
Mean			**−36.59**	**−9.89**
Standard deviation			**0.04**	**0.06**
Standard error			**0.02**	**0.02**
R^2		**0.9997**		

(Table 2). Although lower than the precision of the Prism, the automated system surpassed the company target of 0.5‰ standard deviation. The system was accurate, with no significant difference between the mean value of the automated system and dual inlet mean of −36.5‰. Similar accuracy was obtained for replicate analyses of the DIC standard, BTW, for which the average $\delta^{13}C$ value was −9.9‰ (n = 10; range −9.8 to −10.2‰) by autosampler as compared with −10.2‰ for a single off-line preparation and analysis by the dual-inlet system. In testing linearity, the $\delta^{13}C$ values of the 2% STD or of the BTW standard did not vary significantly as a function of concentration, over a five-fold range (ANOVA, $P < 0.175$, Table 3). Regression analysis demonstrated a significant linear relationship between peak area and CO_2 concentration of the 2% STD ($r^2 \geq 0.999$) and BTW ($r^2 \geq 0.999$).

Soil respiration $\delta^{13}C$ at Jasper Ridge and Blodgett Forest experiments

In keeping with the requirement for Keeling plot analysis, field samples collected at JRGCE displayed a linear change in $\delta^{13}C$ with $[CO_2]^{-1}$ concentration. The relationship was highly significant in both elevated CO_2 and control plots ($r^2 > 0.99$, $P < 0.001$). The estimated $\delta^{13}C$ of CO_2 efflux was −26.3‰ for the control plot and −36.9‰ for the elevated CO_2 plot (Fig. 3(A)). Plant inputs to the control plots had an isotopic signature of −28.3‰, or 2‰ lighter than its respiration. The $\delta^{13}C$ of plant inputs in the elevated CO_2 plot was approximately −48.8% (Table 1). From Eqn. (3), 48% of the soil respiration was derived from carbon that was fixed under elevated CO_2 and 52% came from decomposition of older organic matter. These measurements were made after the growing season had ended and before new grasses had begun to grow, when root respiration was negligible compared to decomposition fluxes. Thus, these values represent the decomposition of organic matter after one growing season of treatment.

The Blodgett Forest field samples also displayed a linear relationship between $\delta^{13}C$ and $[CO_2]^{-1}$, for both tracer and control plots ($r^2 > 0.99$, $P < 0.001$). The estimated $\delta^{13}C$ of CO_2 efflux was −26.1‰ for the control plot and +95.8‰ in a plot that received ^{13}C-enriched pine needles 61 days prior (Fig. 3(B), Table 1). From Eqn. (3), the fractional abundance of CO_2 from the added substrate was 0.05. The CO_2 efflux rate ($2.8\,\mu mol\,m^{-2}\,s^{-1}$) times F gives a total ^{13}C efflux rate of $0.14\,\mu mol\,m^{-2}\,s^{-1}$. When data were averaged across all field replicates (data not shown), the decay rate depended on litter type (needles vs. fine roots) and substrate depth (O vs. A horizon). For example, the *in situ* decomposition rate of needles was 270% faster than that of fine roots at 61 days.

DIC $\delta^{13}C$ of LBNL groundwater

The DIC samples were collected from shallow groundwater wells extending along the axis of a plume of chlorinated solvents at LBNL. Samples were collected November to December of 1997 and September to December of 1999 and analyzed by both the autosampler system and by traditional off-line preparation and dual-inlet analysis. As shown in Fig. 4, there was very close agreement between the two sets of samples and two methods of analyses, particularly given that there were almost 2 years between sampling events and that measured concentrations of contaminants varied considerably over the period. The results show that DIC in groundwater samples from the core of the plume had relatively high $\delta^{13}C$ values (greater than −20‰) whereas samples from the distal parts of the plume had lower ^{13}C-DIC values (to −25‰). This indicates a general shift from anaerobic to aerobic conditions along the length of the plume, which has significant implications for the likely biodegradation pathways for the chlorinated compounds.

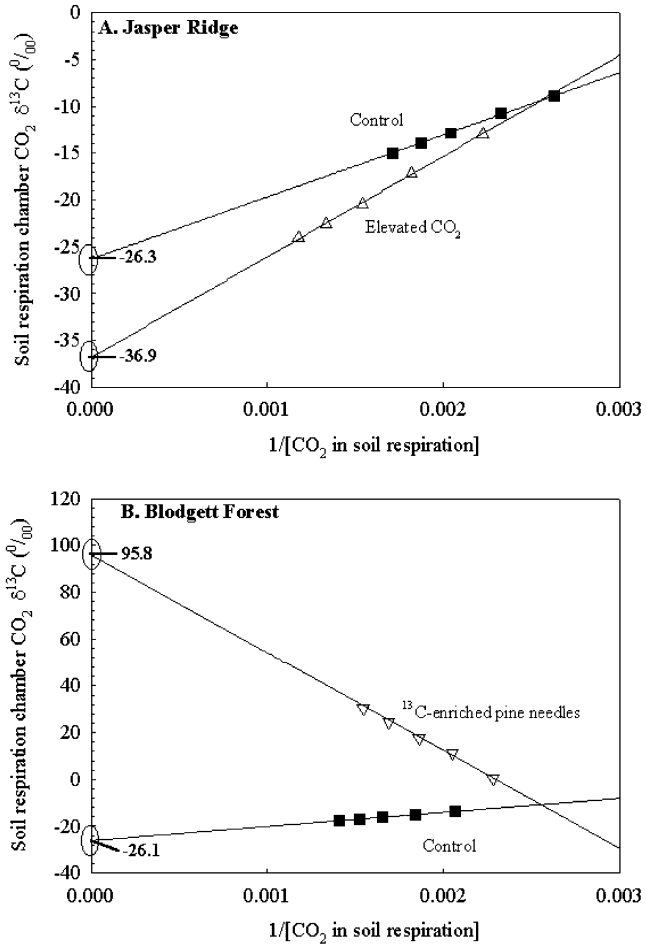

Figure 3. Relationship between the $\delta^{13}C$ of CO_2 in soil respiration chamber and the reciprocal of CO_2 concentration in a soil respiration chamber. The y-intercept of the linear regression, shown in the Keeling plot figure, represents the estimated $\delta^{13}C$ signature of the soil CO_2 efflux. The estimated signature of soil respiration is shown on the graph. The r^2 of each linear regression is $\geq$0.99; (A) Jasper Ridge Global Change Experiment. Samples were collected on 30 October 1999, after one growing season of treatment, from an ambient-CO_2 plot (solid symbols) and a plot with the combined treatment of elevated CO_2, warming, and added nitrogen and precipitation (open symbols); (B) Blodgett Forest Decomposition Experiment. Samples were collected on 16 January 2002 from a control plot (solid symbols), i.e., no ^{13}C-enriched substrate added; and a plot to which ^{13}C-enriched *Pinus ponderosa* needles had been added (open symbols). Samples were taken 61 days after the addition of the labeled substrate to the soil.

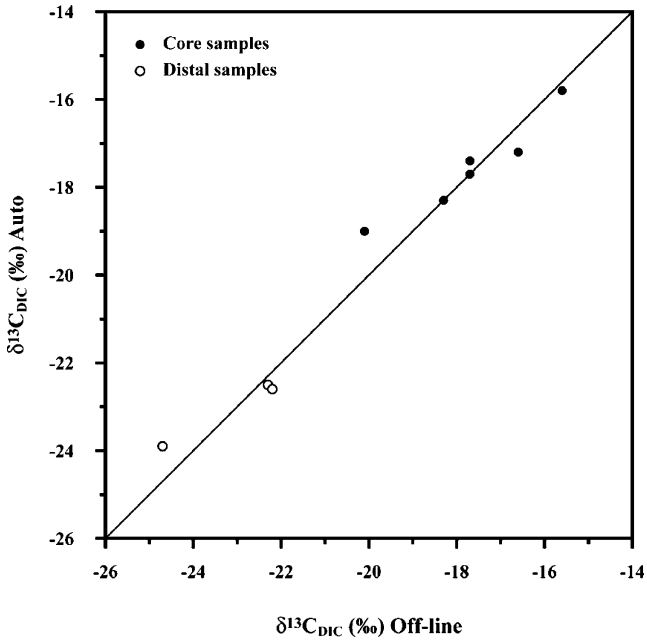

Figure 4. Plot of $\delta^{13}C$ values measured using the headspace autosampler versus an off-line dual-inlet technique. The data are from two sets of samples collected approximately 2 years apart. Solid circles are samples from the central area of a chlorinated solvent plume (within 50 m of source) and open circles are samples from distal areas of the plume.

DISCUSSION

The high precision, accuracy and linearity of the CO_2 and DIC analyses showed that the automated HS40-TG Isoprime system can be used to determine the $\delta^{13}C$ values of CO_2 across a wide range in analyte concentration. Although the degree of accuracy required from such a system is dependent on the application, the observed accuracy is superior to the specifications of the mass spectrometer's manufacturer for similar non-automated systems. For example, Micromass specifies the ability to run five atmospheric CO_2 samples on the trace gas preconcentrator with a $\delta^{13}C$ value not exceeding a standard deviation of 0.5‰; in the current experiment the standard deviation equaled only 0.20‰. Similar accuracy specifications are cited for the trace gas systems manufactured by ThermoFinnigan and PDZ Europa. None of the manufacturers currently specifies the linearity of these systems.

Total system variability must be assessed by including field and laboratory processing. Because the field collection protocols are simple and require no off-line preparation, total variability for this system should compare favorably even with dual-inlet systems that, while analytically precise, require vacuum-line preparation of field samples. In fact, the largest sources of variability and error are likely to be the field collection and laboratory preparation, rather than mass spectrometry.

An improvement of the system described here would be to use a headspace autosampler that is compatible with vials that have a longer shelf life than those tested for this study, and with smaller vials than those used here, allowing for even smaller sample volumes required. For example, LabCo Exetainers[27] come in many sizes and have demonstrated a long sample storage shelf life. They are compatible, for example, with a Gilson autosampler. Alternatively, a flask interface (see, e.g., Ref. 24), offering long storage times, would be ideal if the system capacity and flask design could be modified to handle smaller volumes and large numbers of samples.

The field collection methods performed well with both natural abundance and tracer ^{13}C (both depleted and highly enriched ^{13}C). Changes in $\delta^{13}CO_2$ within soil respiration

chambers were consistent with changes in CO_2 concentration. Estimates of the $\delta^{13}C$ of CO_2 efflux from the experimental sites via Keeling plot analysis had correlation coefficients, $r^2 > 0.99$. A regression coefficient as high as these would be difficult to achieve with Keeling plots made from multiple heights above the canopy (see, e.g., Ref. 8) or from plots made by circulating air within a closed jar (see, e.g., Ref. 25). As expected, the $\delta^{13}C$ values in respiration from the Jasper Ridge elevated CO_2 plots were significantly lower than those from the control plots, reflecting fossil CO_2 input in the elevated CO_2 plots. Similarly, the $^{13}CO_2$ from labeled pine plots provided an estimate of the relative decomposition rates of the two pine substrates and the effect of the soil environment. The results of the analyses of the groundwater DIC samples using the automated system were very reproducible compared with the off-line preparation technique followed by analysis on the dual-inlet Prism.

With the automated system described in this study it is possible to perform large-scale carbon isotope analyses for field experiments. This constitutes a useful tool for studying a wide range of processes in ecological and earth sciences, including carbon cycling and the fate and transport of contaminants.

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